

- 1c. Nucleated blood:** Pipet 20 µl proteinase K into a 1.5 ml or 2 ml microcentrifuge tube. Add 5–10 µl anticoagulant-treated blood. Adjust volume to 220 µl with PBS. Proceed to step 2.
- 1d. Cultured cells:** Centrifuge a maximum of 5×10^6 cells for 5 min at $300 \times g$ (190 rpm). Resuspend in 200 µl PBS. Add 20 µl proteinase K. Proceed to step 2.
2. Add 200 µl Buffer AL. Mix thoroughly by vortexing. Incubate blood samples at 56°C for 10 min.
3. Add 200 µl ethanol (96–100%). Mix thoroughly by vortexing.
4. Pipet the mixture into a DNeasy Mini spin column placed in a 2 ml collection tube. Centrifuge at $\geq 6000 \times g$ (8000 rpm) for 1 min. Discard the flow-through and collection tube.
5. Place the spin column in a new 2 ml collection tube. Add 500 µl Buffer AW1. Centrifuge for 1 min at $\geq 6000 \times g$. Discard the flow-through and collection tube.
6. Place the spin column in a new 2 ml collection tube, add 500 µl Buffer AW2 and centrifuge for 3 min at $20,000 \times g$ (14,000 rpm). Discard the flow-through and collection tube.
7. Transfer the spin column to a new 1.5 ml or 2 ml microcentrifuge tube.
8. Elute the DNA by adding 200 µl Buffer AE to the center of the spin column membrane. Incubate for 1 min at room temperature (15–25°C). Centrifuge for 1 min at $\geq 6000 \times g$.
9. **Optional:** Repeat step 8 for increased DNA yield.

se puede hacer
en 2 pasos de 100 µl



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